

Protein Tyrosine Kinase Inhibitors

Field of the Invention

5 The present invention relates to compounds which inhibit, regulate and/or modulate tyrosine kinase signal transduction and methods of using such compounds to treat tyrosine kinase-dependent diseases and conditions in mammals.

Background of the Invention

10 Throughout this application, various references are cited in parentheses to describe more fully the state of the art to which this invention pertains. The disclosure of these references are hereby incorporated by reference into the present disclosure.

15 Tyrosine kinases are a class of enzymes that catalyze the transfer of the terminal phosphate of adenosine triphosphate to tyrosine residues in protein substrates. Tyrosine kinases are believed, by way of substrate phosphorylation, to play critical roles in signal transduction for a number of cell functions. Though the exact mechanisms of signal transduction is still unclear, tyrosine kinases have been shown to be important contributing
20 factors in cell proliferation, carcinogenesis and cell differentiation.

Tyrosine kinases can be categorized as receptor type or non-receptor type. Receptor type tyrosine kinases have an extracellular, a transmembrane, and an intracellular portion, while non-receptor type tyrosine kinases are wholly intracellular.

25 The receptor-type tyrosine kinases are comprised of a large number of transmembrane receptors with diverse biological activity. Approximately, 20 different subfamilies of receptor-type tyrosine kinases have been identified. One tyrosine kinase subfamily is comprised of EGFR, HER2, HER3, and HER4. Ligands of this subfamily of receptors include epithelial growth factor,
30 TGF- α , amphiregulin, HB-EGF, betacellulin and heregulin. Another subfamily of these receptor-type tyrosine kinases is the insulin subfamily, which includes INS-R, IGF-IR, and IR-R. The PDGF subfamily includes the PDGF- α and β

receptors, CSFIR, c-kit and FLK-II. The FLK family is comprised of the kinase insert domain receptor (KDR), fetal liver kinase-1 (FLK-1), fetal liver kinase-4 (FLK-4) and the fms-like tyrosine kinase-1 (flt-1) (Plowman et al., DN&P 7(6):334-339, 1994, which is hereby incorporated by reference).

5 The non-receptor type of tyrosine kinases are also comprised of numerous subfamilies, including Src, Frk, Btk, Csk, Abl, Zap70, Fes/Fps, Fak, Jak, Ack, and LIMK. Each of these subfamilies is further sub-divided into varying receptors. For example, the Src subfamily is one of the largest and includes Src, Yes, Fyn, Lyn, Lck, Blk, Hck, Fgr, and Yrk. The Src subfamily of
10 enzymes has been linked to oncogenesis (Bolen Oncogene, 8:2025-2031 (1993), which is hereby incorporated by reference).

 Both receptor-type and non-receptor type tyrosine kinases are implicated in cellular signalling pathways leading to numerous pathogenic conditions, including a variety of cancers. For example, the Bcr-Abl tyrosine
15 kinase is the constitutive abnormal tyrosine kinase created by the Philadelphia chromosome abnormality in chronic myeloid leukemia (CML). Inappropriate Bcr-Abl activity is also demonstrated in murine myeloid cells as well as Bcr-Abl positive leukemia lines derived from CML patients in blast crisis.

 Drug screening has been used *in vitro* to try and identify new
20 compounds with potential cell line-specific anti-tumour activity. Such a screening was published with respect to the compound N,N'-bis[4-(1,4,5,6-tetrahydro-5-methyl-2-pyrimidinyl)phenyl]-2,5-pyridinedicarboxamide dihydrochloride (also known as 4'4''-bis(1,4,5,6-tetrahydro-5-methyl-2-pyrimidinyl)-2,5-pyridinedicarboxanilide dihydrochloride trihydrate) (National
25 Cancer Institute 1965) which was not identified as a protein tyrosine kinase inhibitor. The screening conducted using a leukemia mouse model was not conclusive.

 It has now been identified that the aforementioned compound herein referred to as "COTI-001" is involved in the signal transduction of tyrosine
30 kinases and in particular those tyrosine kinases involved in various malignancies leading to the use of such a compound in methods to effectively treat a variety of cancers in mammals.

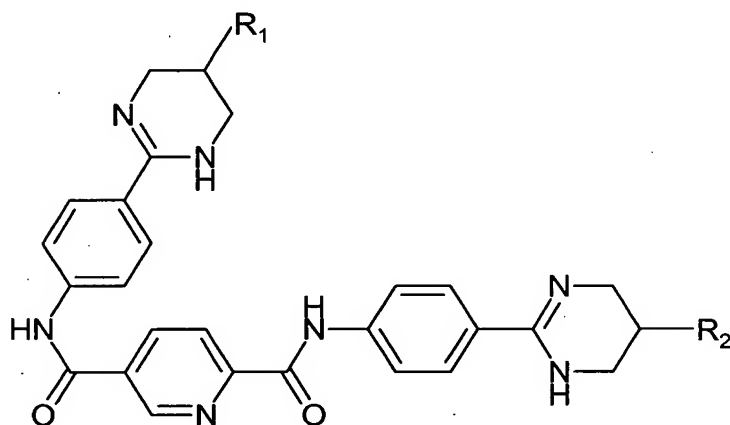
Summary of the Invention

The present invention relates to the identification of the role of COTI-001 to inhibit, regulate and/or modulate tyrosine kinase signal transduction to treat tyrosine kinase-dependent diseases and conditions, such as cancer and tumour growth, and the like in mammals.

More particularly, the present invention relates to COTI-001 and related compounds that are capable of inhibiting, modulating and/or regulating signal transduction of both receptor-type and non-receptor type tyrosine kinases.

The compounds are novel protein tyrosine kinase inhibitors useful in the treatment of a variety of malignancies involving inappropriate tyrosine kinase activity.

According to one aspect of the present invention, there is provided a compound of Formula I, and the pharmaceutically acceptable salts thereof and stereoisomers thereof:

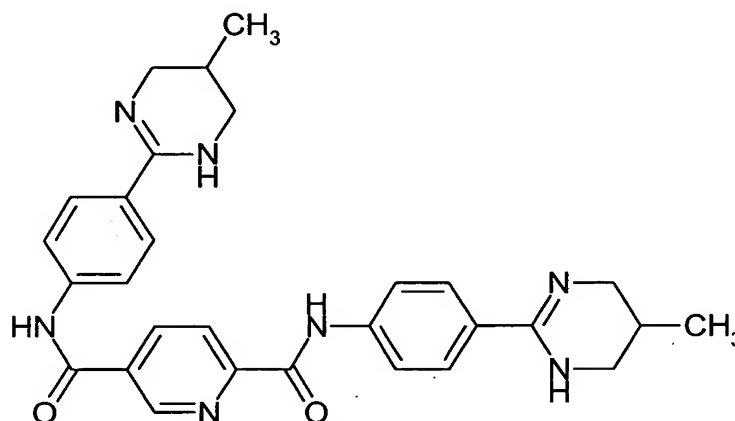


Formula I

wherein R_1 and R_2 are independently selected from the group consisting of H; alkyl; alkenyl; alkynyl; halogen; aryl; heteroaryl containing N, O, or S; the aryl and heteroaryl may be further substituted with halogen, an alkyl, alkenyl, and alkynyl; NZ_1Z_2 , wherein Z_1 and Z_2 are independently selected from the

group consisting of H and alkyl; and (CO)Y wherein Y is selected from the group consisting of H, alkyl, alkenyl, alkynyl, aryl, heteroaryl containing N, O, or S, and the aryl and heteroaryl may be further substituted with halogen, alkyl, alkenyl, and alkynyl.

- 5 In one aspect of the present invention, the compound is represented as Formula II:

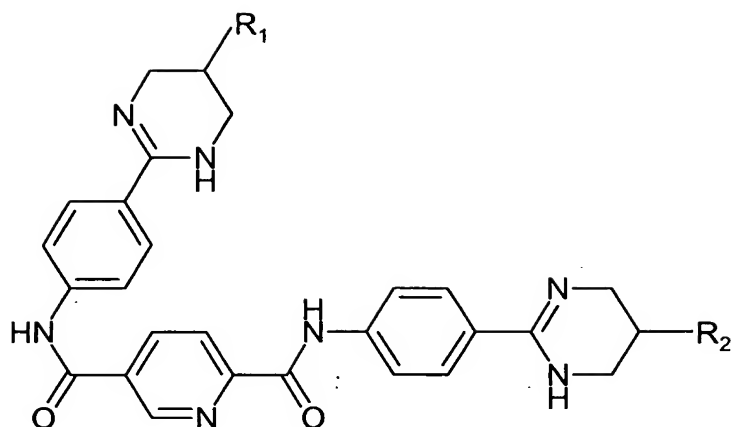


Formula II

- According to another aspect of the present invention is a pharmaceutical composition which is comprised of a compound in accordance with formula I together with a pharmaceutically acceptable carrier.
- 10

According to another aspect of the present invention is a pharmaceutical composition which is comprised of a compound in accordance with formula II together with a pharmaceutically acceptable carrier.

- According to another aspect of the present invention, there is provided a composition comprising a compound selected from the group consisting of Formula I, a pharmaceutically acceptable salt thereof, a stereoisomer thereof, and mixtures thereof:
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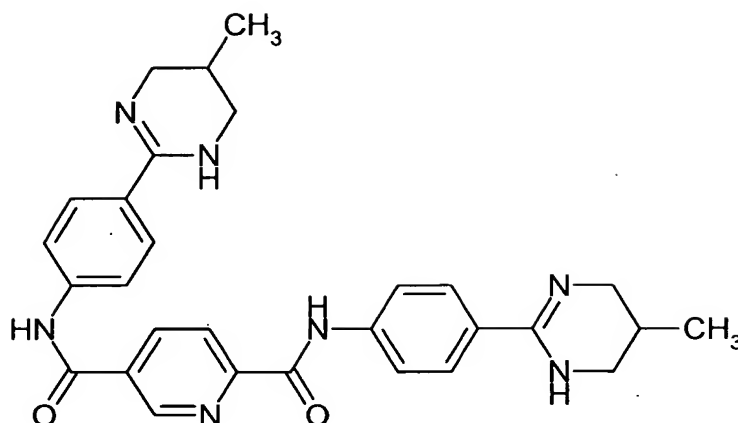


Formula I

wherein R_1 and R_2 are independently selected from the group consisting of H; alkyl; alkenyl; alkynyl; halogen; aryl; heteroaryl containing N, O, or S; the aryl and heteroaryl may be further substituted with halogen, an alkyl, alkenyl, and alkynyl; NZ_1Z_2 , wherein Z_1 and Z_2 are independently selected from the group consisting of H and alkyl; and (CO)Y wherein Y is selected from the group consisting of H, alkyl, alkenyl, alkynyl, aryl, heteroaryl containing N, O, or S; with the proviso that when R_1 is hydrogen, R_2 is a group other than hydrogen; and a pharmaceutically acceptable carrier, wherein the composition is for treatment of cancer involving inappropriate tyrosine kinase activity.

According to another aspect of the present invention, there is provided a composition as above, with the proviso that when R_1 is methyl, R_2 is a group other than methyl.

According to yet another aspect of the present invention, there is provided a composition comprising a compound selected from the group consisting of Formula II, a pharmaceutically acceptable salt thereof, a stereoisomer thereof and mixtures thereof:

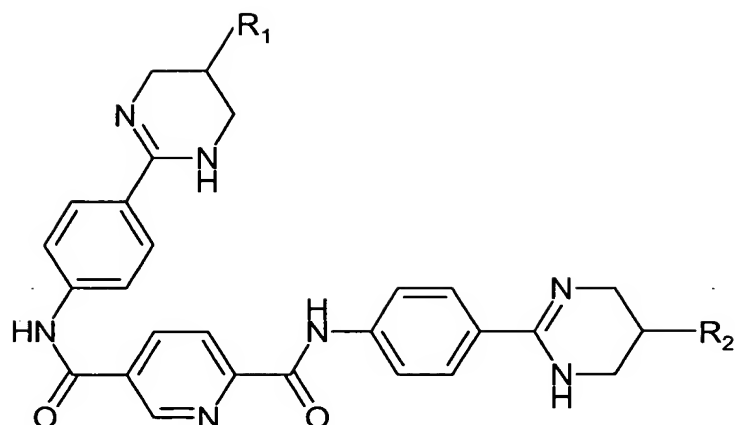


Formula II

and a pharmaceutically acceptable carrier, wherein the composition is for treatment of cancer involving inappropriate tyrosine kinase activity.

According to another aspect of the present invention is a method of
 5 treating or preventing cancer involving inappropriate tyrosine kinase activity in a mammal in need of such treatment which is comprised of administering to the mammal a therapeutically effective amount of a compound selected from the group consisting of a compound of Formula I and/or II, a pharmaceutically acceptable salt thereof, a stereoisomer thereof, and mixtures thereof.

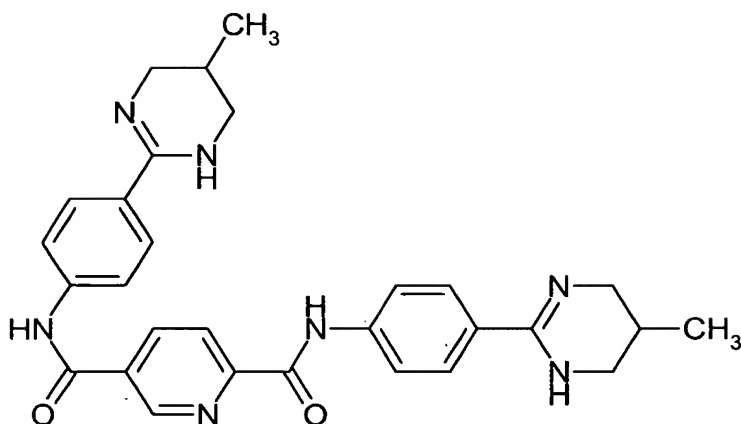
10 According to another aspect of the present invention, there is provided a method for treatment of cancer involving inappropriate tyrosine kinase activity in a mammal in need of such treatment, the method comprising administering to the mammal a therapeutically effective amount of a compound selected from the group consisting of Formula I, a pharmaceutically acceptable salt
 15 thereof, a stereoisomer thereof, and mixtures thereof:



Formula I

wherein R_1 and R_2 are independently selected from the group consisting of H; alkyl; alkenyl; alkynyl; halogen; aryl; heteroaryl containing N, O, or S; the aryl and heteroaryl may be further substituted with halogen, an alkyl, alkenyl, and alkynyl; NZ_1Z_2 , wherein Z_1 and Z_2 are independently selected from the group consisting of H and alkyl; and $(CO)Y$ wherein Y is selected from the group consisting of H, alkyl, alkenyl, alkynyl, aryl, heteroaryl containing N, O, or S; with the proviso that when R_1 is hydrogen, R_2 is a group other than hydrogen.

According to yet another aspect of the present invention, there is provided the method for treatment of cancer involving inappropriate tyrosine kinase activity as above, wherein the compound is selected from the group consisting of Formula II, a pharmaceutically acceptable salt thereof, a stereoisomer thereof and mixtures thereof:



Formula II

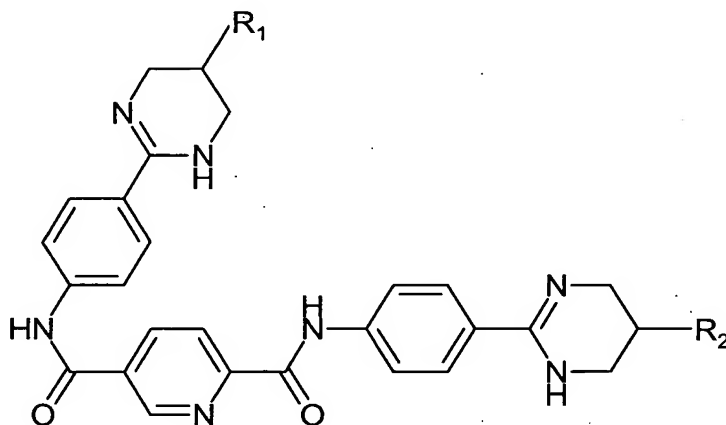
According to another aspect of the present invention is a method of treating cancer or preventing cancer using a composition comprising a compound selected from the group consisting of Formula I and/or II, a pharmaceutically acceptable salt thereof, a stereoisomer thereof, and mixtures thereof, wherein the cancer is selected from cancers of the breast, leukemias, melanoma, stomach, colon, central nervous system (CNS), ovarian and prostate and those listed in Table I.

According to still another aspect of the present invention is a method of treating or preventing cancer comprising administration of a therapeutically effective amount of a composition comprising a compound selected from the group consisting of Formula I and/or II, a pharmaceutically acceptable salt thereof, a stereoisomer thereof, and mixtures thereof, wherein the cancer is a leukemia including chronic myelogenous leukemia (CML), acute myelogenous leukemia (AML) and acute lymphoblastic leukemia (ALL).

According to another aspect of the present invention is a method of treating or preventing a tyrosine kinase-dependent disease or condition which comprises administering a therapeutically effective amount of a compound selected from the group consisting of Formula I and/or II, a pharmaceutically acceptable salt thereof, a stereoisomer thereof, and mixtures thereof.

According to another aspect of the present invention is the use of a compound selected from the group consisting of Formula I and/or II, a pharmaceutically acceptable salt thereof, a stereoisomer thereof, and mixtures thereof in a medicament for the treatment of a disease condition involving inappropriate tyrosine kinase activity.

According to another aspect of the present invention, there is provided a use of a compound selected from the group consisting of Formula I, a pharmaceutically acceptable salt thereof, a stereoisomer thereof, and mixtures thereof:



Formula I

10

wherein R_1 and R_2 are independently selected from the group consisting of H; alkyl; alkenyl; alkynyl; halogen; aryl; heteroaryl containing N, O, or S; the aryl and heteroaryl may be further substituted with halogen, an alkyl, alkenyl, and alkynyl; NZ_1Z_2 , wherein Z_1 and Z_2 are independently selected from the group consisting of H and alkyl; and $(CO)Y$ wherein Y is selected from the group consisting of H, alkyl, alkenyl, alkynyl, aryl, heteroaryl containing N, O, or S; with the proviso that when R_1 is hydrogen, R_2 is a group other than hydrogen, in a medicament for the treatment of cancer involving inappropriate tyrosine kinase activity in a mammal in need of such treatment.

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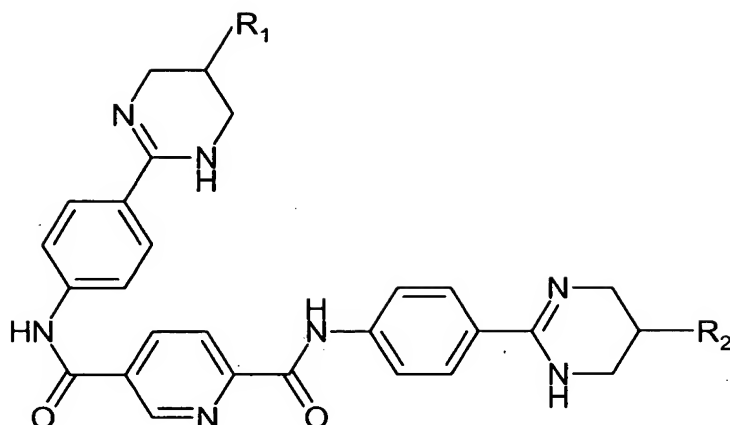
According to still a further aspect of the present invention is a method for the treatment of a tyrosine-kinase dependent disease or condition comprising administering a therapeutically effective amount of a composition comprising a compound selected from the group consisting of Formula I
5 and/or II, a pharmaceutically acceptable salt thereof, a stereoisomer thereof, and mixtures thereof, further comprising a second compound selected from the group consisting of an estrogen receptor modulator, an androgen receptor modulator, retinoid receptor modulator, a cytotoxic agent, an anti-proliferative agent, a tyrosine kinase inhibitor, an inhibitor of epidermal-derived growth
10 factor, an inhibitor of fibroblast-derived growth factor, an inhibitor of platelet derived growth factor, an MMP inhibitor, an integrin blocker, interferon- α , interleukin-12, pentosan polysulfate, a cyclooxygenase inhibitor, carboxyamidotriazole, combretastatin A-4, squalamine, 6-O-chloroacetyl-carbonyl)-fumagillol, thalidomide, angiostatin, and troponin-1, tamoxifen and
15 raloxifene.

According to a further aspect of the present invention is a method of treating cancer which comprises administering a therapeutically effective amount of a compound selected from the group consisting of Formula I and/or II, a pharmaceutically acceptable salt thereof, a stereoisomer thereof, and
20 mixtures thereof, in combination with a therapy selected from the group consisting of radiation therapy and chemotherapy.

Other features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while
25 indicating embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from the detailed description.

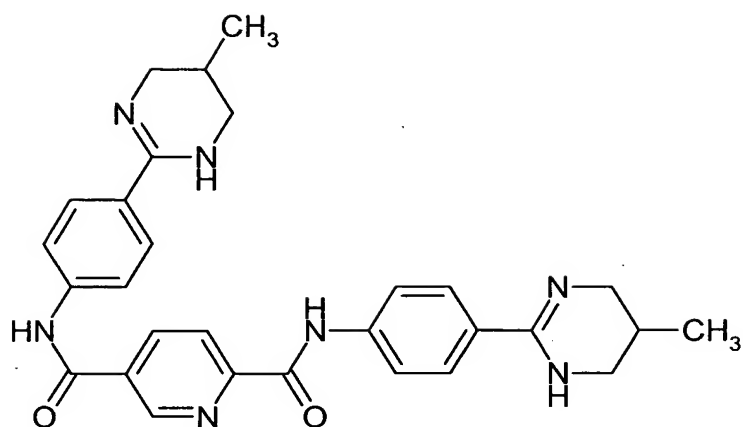
30 Detailed Description of the Preferred Embodiments

The compound of the invention is illustrated by Formula I:

Formula I

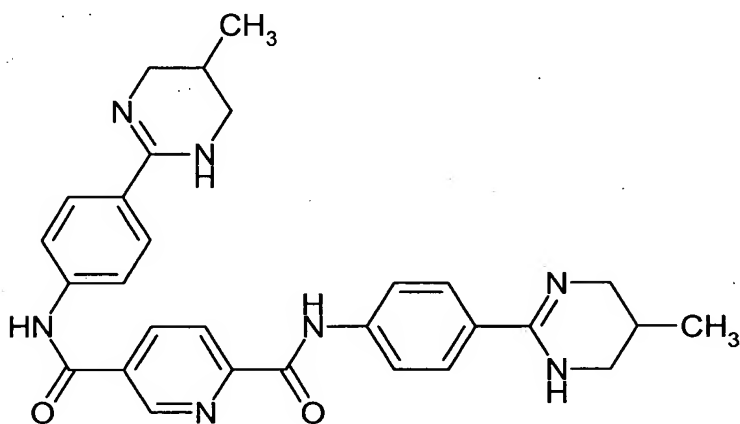
wherein R_1 and R_2 are independently selected from the group consisting of H; alkyl; alkenyl; alkynyl; halogen; aryl; heteroaryl containing N, O, or S; the aryl and heteroaryl may be further substituted with halogen, an alkyl, alkenyl, and alkynyl; NZ_1Z_2 , wherein Z_1 and Z_2 are independently selected from the group consisting of H and alkyl; and $(CO)Y$ wherein Y is selected from the group consisting of H, alkyl, alkenyl, alkynyl, aryl, heteroaryl containing N, O, or S, and the aryl and heteroaryl may be further substituted with halogen, alkyl, alkenyl, and alkynyl, with the proviso that when R_1 is hydrogen, R_2 is a group other than hydrogen.

In one embodiment of the invention, the compound has the formula:

Formula II

The invention is directed to the use of the compound of Formula I and II, as well as its' pharmaceutically acceptable salts and stereoisomers thereof
 5 in methods for treating cancer. More specifically, in methods for treating cancer involving inappropriate tyrosine kinase activity.

Using an in silico assay, the COTI-001 compound of Formula II:

Formula II

was predicted to have *in vitro* activity against a variety of cancerous cell types shown in Table 1. Also, while not explicitly shown, the COTI-001 compound of Formula II was also predicted to have *in vitro* activity against HIV. In addition, Example 2 shows *in vitro* activity of Formula II against a variety of cancerous cell types. This example demonstrates that the compound of Formula II is effective in the treatment of such cancers.

Included within the scope of the present invention is a pharmaceutical composition which is comprised of Formula I and/or II, a pharmaceutically acceptable salt thereof, a stereoisomer thereof, and mixtures thereof as described above and a pharmaceutically acceptable carrier. The present invention also encompasses a method of treating or preventing cancer in a mammal in need of such treatment which is comprised of administering to the mammal a therapeutically effective amount of a compound of Formula I and/or II, a pharmaceutically acceptable salt thereof, a stereoisomer thereof, and mixtures thereof. Preferred cancers for treatment are selected from cancers of the breast, colon, prostate, gastric, melanoma, ovarian and leukemias. Another preferred form of cancer is chronic myeloid leukemia (CML).

The invention also encompasses pharmaceutical compositions comprising a compound selected from the group consisting of Formula I and/or II, a pharmaceutically acceptable salt thereof, a stereoisomer thereof, and mixtures thereof for the treatment of HIV.

The compositions and methods of the invention can include a compound selected from the group consisting of Formula I and/or II, a pharmaceutically acceptable salt thereof, a stereoisomer thereof, and mixtures thereof, as desired.

Also included is a method of treating or preventing a tyrosine kinase-dependent disease or condition in a mammal which comprises administering to a mammalian patient in need of such treatment a therapeutically effective amount of a compound selected from the group consisting of Formula I and/or II, a pharmaceutically acceptable salt thereof, a stereoisomer thereof, and mixtures thereof. The therapeutic amount varies according to the specific

disease and is discernible to the skilled artisan without undue experimentation.

Also included in the scope of the invention is a method of treating cancer which comprises administering a therapeutically effective amount of a compound selected from the group consisting of Formula I and/or II, a pharmaceutically acceptable salt thereof, a stereoisomer thereof, and mixtures thereof, in combination with radiation therapy and/or in combination with a compound generally known to for use in selected cancers and selected from the group consisting of an estrogen receptor modulator, an androgen receptor modulator, retinoid receptor modulator, a cytotoxic agent and an antiproliferative agent. These and other aspects of the invention will be apparent from the teachings contained herein.

"Tyrosine kinase-dependent diseases or conditions" refers to pathologic conditions that depend on the activity of one or more tyrosine kinases. Tyrosine kinases either directly or indirectly participate in the signal transduction pathways of a variety of cellular activities including proliferation, adhesion and migration, and differentiation. Diseases associated with tyrosine kinase activities include but are not limited to the proliferation of tumor cells.

The compounds of the present invention may have asymmetric centers, chiral axes, and chiral planes (as described in: E. L. Eliel and S. H. Wilen, Stereo-chemistry of Carbon Compounds, John Wiley & Sons, New York, 1994, pages 1119-1190), and occur as racemates, racemic mixtures, and as individual diastereomers, with all possible isomers and mixtures thereof, including optical isomers, being included in the present invention. In addition, the compounds disclosed herein may exist as tautomers and both tautomeric forms are intended to be encompassed by the scope of the invention, even though only one tautomeric structure is depicted.

As used herein, "alkyl" is intended to include both branched, straight-chain, and cyclic saturated aliphatic hydrocarbon groups having the specified number of carbon atoms. For example, C₁-C₁₀, as in "C₁₋₁₀ alkyl" is defined to include groups having 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 carbons in a linear, branched, or cyclic arrangement. For example, "C₁-C₁₀ alkyl" specifically

includes methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, and so on, as well as cycloalkyls such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, tetrahydro-naphthalene, methylenecyclohexyl, and so on. "Alkoxy" represents an alkyl group of indicated number of carbon atoms attached
5 through an oxygen bridge.

If no number of carbon atoms is specified, the term "alkenyl" refers to a non-aromatic hydrocarbon radical, straight, branched or cyclic, containing from 2 to 10 carbon atoms and at least one carbon to carbon double bond. Preferably one carbon to carbon double bond is present, and up to 4 non-
10 aromatic carbon-carbon double bonds may be present. Thus, "C₂-C₆ alkenyl" means an alkenyl radical having from 2 to 6 carbon atoms. Alkenyl groups include ethenyl, propenyl, butenyl and cyclohexenyl. As described above with respect to alkyl, the straight, branched or cyclic portion of the alkenyl group may contain double bonds and may be substituted if a substituted alkenyl
15 group is indicated.

The term "alkynyl" refers to a hydrocarbon radical straight, branched or cyclic, containing from 2 to 10 carbon atoms and at least one carbon to carbon triple bond. Up to 3 carbon-carbon triple bonds may be present. Thus, "C₂-C₆ alkynyl" means an alkynyl radical having from 2 to 6 carbon atoms.
20 Alkynyl groups include ethynyl, propynyl and butynyl. As described above with respect to alkyl, the straight, branched or cyclic portion of the alkynyl group may contain triple bonds and may be substituted if a substituted alkynyl group is indicated.

As used herein, "aryl" is intended to mean any stable monocyclic or
25 bicyclic carbon ring of up to 7 atoms in each ring, wherein at least one ring is aromatic. Examples of such aryl elements include phenyl, naphthyl, tetrahydro-naphthyl, indanyl, biphenyl, phenanthryl, anthryl or acenaphthyl. In cases where the aryl substituent is bicyclic and one ring is non-aromatic, it is understood that attachment is via the aromatic ring.

30 The term heteroaryl, as used herein, represents a stable monocyclic or bicyclic ring of up to 7 atoms in each ring, wherein at least one ring is aromatic and contains from 1 to 4 heteroatoms selected from the group

consisting of O, N and S. Heteroaryl groups within the scope of this definition include but are not limited to: acridinyl, carbazolyl, cinnolinyl, quinoxalinyl, pyrrazolyl, indolyl, benzotriazolyl, furanyl, thienyl, benzothienyl, benzofuranyl, quinolinyl, isoquinolinyl, oxazolyl, isoxazolyl, indolyl, pyrazinyl, pyridazinyl, 5 pyridinyl, pyrimidinyl, pyrrolyl, tetrahydroquinoline. In cases where the heteroaryl substituent is bicyclic and one ring is non-aromatic or contains no heteroatoms, it is understood that attachment is via the aromatic ring or via the heteroatom containing ring, respectively.

As appreciated by those of skill in the art, "halo" or "halogen" as used 10 herein is intended to include chloro, fluoro, bromo and iodo. The term "heterocycle" or "heterocyclyl" as used herein is intended to mean a 5- to 10-membered aromatic or nonaromatic heterocycle containing from 1 to 4 heteroatoms selected from the group consisting of O, N and S, and includes bicyclic groups. "Heterocyclyl" therefore includes the above mentioned 15 heteroaryls, as well as dihydro and tetrahydro analogs thereof. Further examples of "heterocyclyl" include, but are not limited to the following: benzoimidazolyl, benzofuranyl, benzofurazanyl, benzopyrazolyl, benzotriazolyl, benzothiophenyl, benzoxazolyl, carbazolyl, carbolinyl, cinnolinyl, furanyl, imidazolyl, indolinyl, indolyl, indolaziny, indazolyl, 20 isobenzofuranyl, isoindolyl, isoquinolyl, isothiazolyl, isoxazolyl, naphthpyridinyl, oxadiazolyl, oxazolyl, oxazoline, isoxazoline, oxetanyl, pyranyl, pyrazinyl, pyrazolyl, pyridazinyl, pyridopyridinyl, pyridazinyl, pyridyl, pyrimidyl, pyrrolyl, quinazolinyl, quinolyl, quinoxalinyl, tetrahydropyranyl, tetrazolyl, tetrazolopyridyl, thiadiazolyl, thiazolyl, thienyl, triazolyl, azetidiny, 25 1,4-dioxanyl, hexahydroazepinyl, piperazinyl, piperidinyl, pyrrolidinyl, morpholinyl, thiomorpholinyl, dihydrobenzoimidazolyl, dihydrobenzofuranyl, dihydrobenzothiophenyl, dihydrobenzoxazolyl, dihydrofuranyl, dihydroimidazolyl, dihydroindolyl, dihydroisooxazolyl, dihydroisothiazolyl, dihydrooxadiazolyl, dihydrooxazolyl, dihydropyrazinyl, dihydropyrazolyl, 30 dihydropyridinyl, dihydropyrimidinyl, dihydropyrrolyl, dihydroquinolinyl, dihydrotetrazolyl, dihydrothiadiazolyl, dihydrothiazolyl, dihydrothienyl,

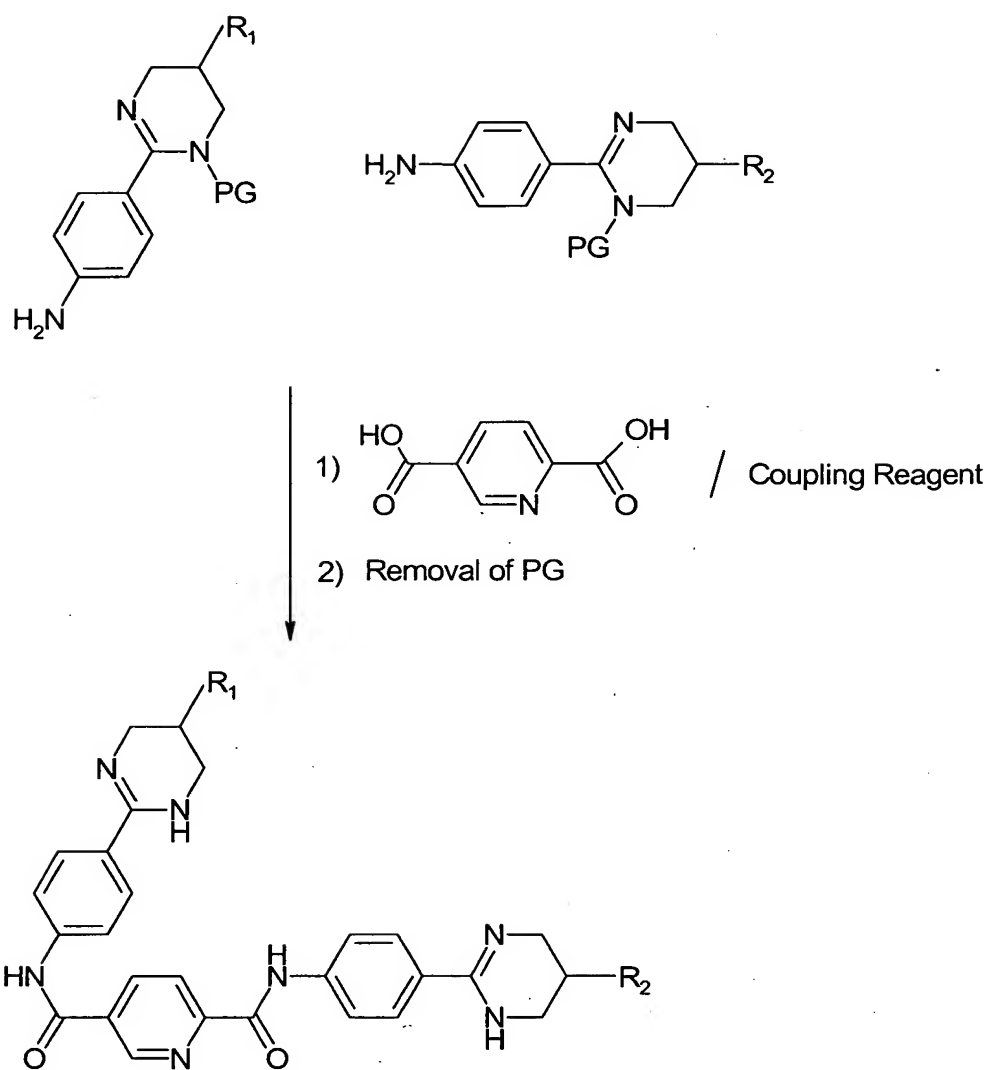
dihydrotriazolyl, dihydroazetidynyl, methylenedioxybenzoyl, tetrahydrofuranyl, and tetrahydrothienyl, and N-oxides thereof.

The pharmaceutically acceptable salts of the compounds of this invention include the conventional non-toxic salts of the compounds of this invention as formed, e.g., from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxy-benzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, trifluoroacetic and the like.

The pharmaceutically acceptable salts of the compounds of this invention can be synthesized from the compounds of this invention which contain a basic or acidic moiety by conventional chemical methods. Generally, the salts of the basic compounds are prepared either by ion exchange chromatography or by reacting the free base with stoichiometric amounts or with an excess of the desired salt-forming inorganic or organic acid in a suitable solvent or various combinations of solvents. Similarly, the salts of the acidic compounds are formed by reactions with the appropriate inorganic or organic base.

The compounds of this invention may be prepared by employing reactions and standard manipulations that are known in the literature or exemplified in the experimental procedures.

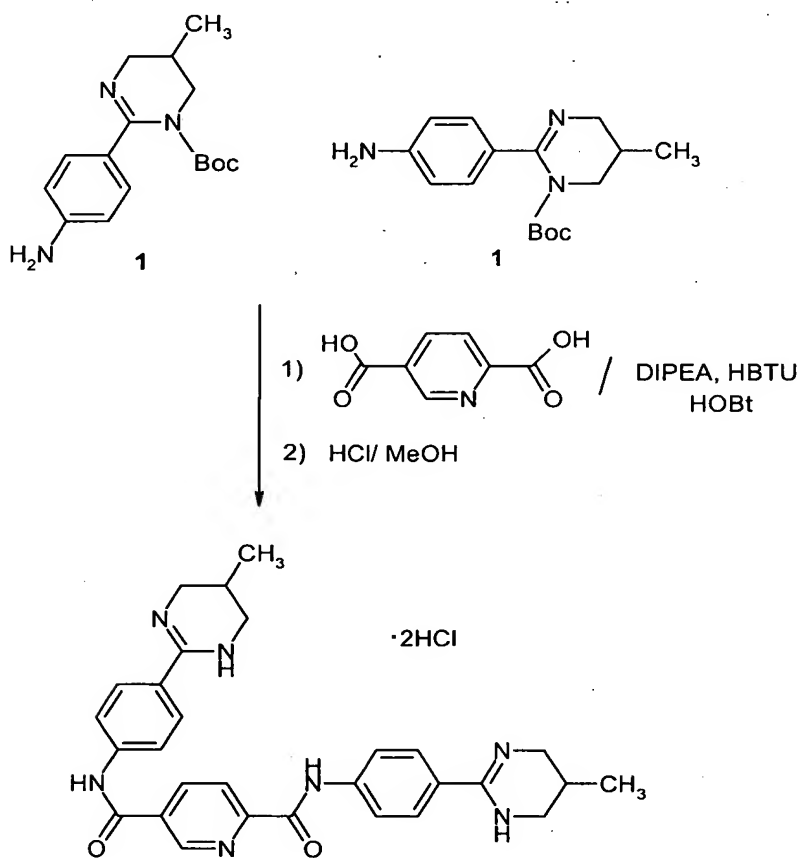
The compound of general Formula I may be synthesized using a coupling reaction as follows:



PG= Protecting Group

A suitable coupling catalyst for promoting diamide bond formation is, but not limited to, a mixture of HBTU (O-Benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate) or HATU (O-(7-Azabenzotriazole-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate); DIPEA (N,N-diisopropylethylamine); and HOBt (1-hydroxybenzotriazole). As a result of deprotection, the salt of Formula I may be formed. The free base may be obtained by treating the salt with a base such as, but not limited to, sodium hydroxide and sodium carbonate.

In an illustrative example the salt of Formula II may be synthesized as shown in Scheme 1.

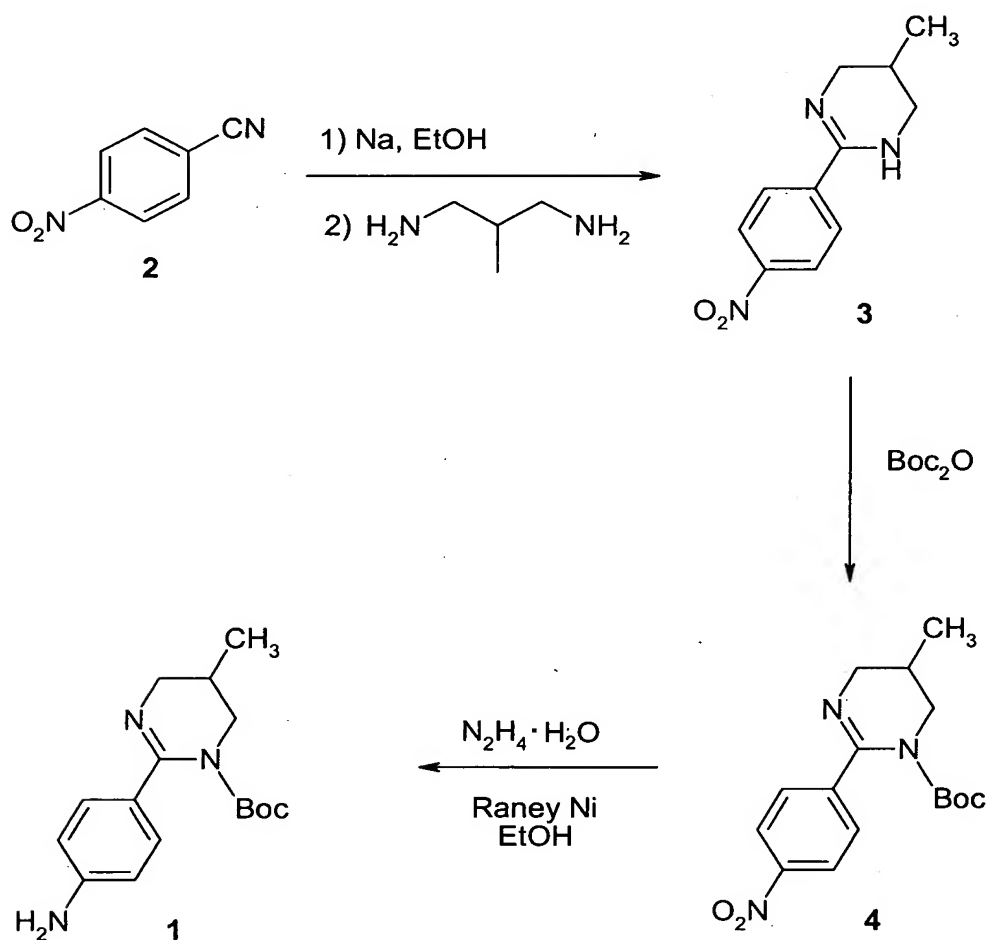


Formula II

Scheme 1

Intermediate **1** underwent a coupling reaction in the presence of 2,5-pyridinedicarboxylic acid and HBTU and DIPEA in HOBT to yield the Boc-protected coupling product. The Boc protective groups were removed with saturated HCl solution in MeOH to afford the salt of Formula I.

- 5 Intermediates for the coupling reaction may be prepared using various reactions known in the literature. For instance, intermediate **1** may be synthesized as shown in Scheme 2.



Scheme 2

For the transformation of 4-nitrobenzonitrile **2** into the pyrimidine **3** was conducted using a similar procedure as described in European Patent Application 0225725 A1; incorporated herein by reference. The pyrimidine **3** was protected using Boc_2O (di-tert-butyl dicarbonate) to yield Boc-protected
5 compound **4**, which was then reduced to intermediate **1**.

The aforementioned compounds selected from the group consisting of Formula I and/or II, a pharmaceutically acceptable salt thereof, a stereoisomer thereof, and mixtures thereof are useful as pharmaceutical agents for mammals, especially for humans, in the treatment of tyrosine kinase
10 dependent diseases and in particular the treatment of various cancers.

The compounds selected from the group consisting of Formula I and/or II, a pharmaceutically acceptable salt thereof, a stereoisomer thereof, and mixtures thereof may be administered to patients for use in the treatment of cancer.

15 The compounds selected from the group consisting of Formula I and/or II; a pharmaceutically acceptable salt thereof, a stereoisomer thereof, and mixtures thereof may be administered to mammals, preferably humans, either alone or, preferably, in combination with pharmaceutically acceptable carriers or diluents, optionally with known adjuvants, such as alum, in a
20 pharmaceutical composition, according to standard pharmaceutical practice. The compounds can be administered orally or parenterally, including the intravenous, intramuscular, intraperitoneal, subcutaneous, rectal and topical routes of administration.

For oral use of a chemotherapeutic compound selected from the group
25 consisting of Formula I and/or II, a pharmaceutically acceptable salt thereof, a stereoisomer thereof, and mixtures thereof, the selected compound may be administered, for example, in the form of tablets or capsules, or as an aqueous solution or suspension. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch, and lubricating
30 agents, such as magnesium stearate, are commonly added. For oral administration in capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are required for oral use, the active

ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring agents may be added. For intramuscular, intraperitoneal, subcutaneous and intravenous use, sterile solutions of the active ingredient are usually prepared, and the pH of the solutions should be suitably adjusted and buffered. For intravenous use, the total concentration of solutes should be controlled in order to render the preparation isotonic.

The compounds selected from the group consisting of Formula I and/or II, a pharmaceutically acceptable salt thereof, a stereoisomer thereof, and mixtures thereof may also be co-administered with other well known therapeutic agents that are selected for their particular usefulness against the condition that is being treated.

The compounds selected from the group consisting of Formula I and/or II, a pharmaceutically acceptable salt thereof, a stereoisomer thereof, and mixtures thereof are also useful in combination with known anti-cancer agents. Such known anti-cancer agents include the following: estrogen receptor modulators, androgen receptor modulators, retinoid receptor modulators, cytotoxic agents, antiproliferative agents, prenyl-protein transferase inhibitors, HMG-CoA reductase inhibitors, HIV protease inhibitors, reverse transcriptase inhibitors, and other angiogenesis inhibitors. The instant compounds are particularly useful when coadministered with radiation therapy. The synergistic effects of inhibiting VEGF in combination with radiation therapy have been described in the art. (see WO 00/61186).

"Estrogen receptor modulators" refers to compounds which interfere or inhibit the binding of estrogen to the receptor, regardless of mechanism. Examples of estrogen receptor modulators include, but are not limited to, tamoxifen, raloxifene, idoxifene, LY353381, LY117081, toremifene, fulvestrant, 4-[7-(2,2-dimethyl-1-oxopropoxy-4-methyl-2-[4-[2-(1-piperidiny)ethoxy]ph- enyl]-2H-1-benzopyran-3-yl]-phenyl-2,2-dimethylpropanoate, 4,4'-dihydroxybenzophenone-2,4-dinitrophenyl-hydrazone, and SH646.

"Androgen receptor modulators" refers to compounds which interfere or inhibit the binding of androgens to the receptor, regardless of mechanism. Examples of androgen receptor modulators include finasteride and other 5 α -

reductase inhibitors, nilutamide, flutamide, bicalutamide, liarozole, and abiraterone acetate.

"Retinoid receptor modulators" refers to compounds which interfere or inhibit the binding of retinoids to the receptor, regardless of mechanism.

- 5 Examples of such retinoid receptor modulators include bexarotene, tretinoin, 13-cis-retinoic acid, 9-cis-retinoic acid, α -difluoromethylomithine, ILX23-7553, trans-N-(4'-hydroxyphenyl) retinamide and N-4-carboxyphenyl retinamide.

- "Cytotoxic agents" refer to compounds which cause cell death primarily by interfering directly with the cell's functioning or inhibit or interfere with cell
10 myosis, including alkylating agents, tumor necrosis factors, intercalators, microtubulin inhibitors, and topoisomerase inhibitors.

- Examples of cytotoxic agents include, but are not limited to, tirapazimine, sertenef, cachectin, ifosfamide, tasonermin, lonidamine, carboplatin, altretamine, prednimustine, dibromodulcitol, ranimustine,
15 fotemustine, nedaplatin, oxaliplatin, temozolomide, heptaplatin, estramustine, improsulfan tosilate, trofosfamide, nimustine, dibrospidium chloride, pumitepa, lobaplatin, satraplatin, profiromycin, cisplatin, irofulven, dexifosfamide, cis-aminedichloro(2-methyl-pyridine) platinum, benzylguanine, glufosfamide, GPX100, (trans, trans, trans)-bis-mu-(hexane-1,6-diamine)-mu-[diamine-
20 platinum(II)]bis[diamine(chloro)platinum (II)]tetrachloride, diarizidinylspermine, arsenic trioxide, 1-(11-dodecylamino-10-hydroxyundecyl)-3,7-dimethylxanthine, zorubicin, idarubicin, daunorubicin, bisantrene, mitoxantrone, pirarubicin, pinafide, valrubicin, amrubicin, antineoplaston, 3'-deamino-3'-morpholino- -13-deoxo-10-hydroxycarminomycin, annamycin,
25 galarubicin, elinafide, MEN10755, and 4-demethoxy-3-deamino-3-aziridiny-4-methylsulphonyl-daunor-ubicin (see WO 00/50032).

- Examples of microtubulin inhibitors include paclitaxel, vindesine sulfate, 3',4'-didehydro-4'-deoxy-8'-norvincal leukoblastine, docetaxol, rhizoxin, dolastatin, mivobulin isethionate, auristatin, cemadotin, RPR109881,
30 BMS184476, vinflunine, cryptophycin, 2,3,4,5,6-pentafluoro-N-(- 3-fluoro-4-methoxyphenyl) benzene sulfonamide, anhydrovinblastine, N,N-dimethyl-L-

valyl-L-valyl-N-methyl-L-valyl-L-prolyl-L-proline-t-butylamide, TDX258, and BMS 188797.

Some examples of topoisomerase inhibitors are topotecan, hycaptamine, irinotecan, rubitecan, 6-ethoxypropionyl-3',4'-O-exo-benzylidene-chartreusin, 9-methoxy-N,N-dimethyl-5-nitropyrzolo[3,4,5-kl]acridine-2-(6H)propanamine, 1-amino-9-ethyl-5-fluoro-2,3-dihydro-9-hydroxy-4-methyl-1H,12H benzo[de]pyrano[3',4':b,7]indolizino [1,2b]quinoline-10,13(9H,15H) dione, lurtotecan, 7-[2-(N-isopropylamino)ethyl]-(20S)camptothecin, BNP1350, BNPI1100, BN80915, BN80942, etoposide phosphate, teniposide, sobuzoxane, 2'-dimethylamino-2'-deoxy-etoposide, GL331, N-[2-(dimethylamino)ethyl]-9-hydroxy-5,6-dimethyl-6H-pyrido[4,3-b]carbazole-1-carboxamide, asulacrine, (5a, 5aB, 8aa,9b)-9-[2-[N-[2-(dimethylamino)ethyl]-N-methylamino]ethyl]-5-[4-Hydroxy-3,5-dimethoxyphenyl]-5,5a,6,8,8a,-9-hexohydrofuro(3',4':6,7)naphtho(2,3-d)-1,3-dioxol-6-one, 2,3-(methylenedioxy)-5-methyl-7-hydroxy-8-methoxybenzo[c]-phenanthridinium, 6,9-bis[(2-aminoethyl)amino]benzo[g]isoguinoline-5,10-dione, 5-(3-aminopropylamino)-7,10-dihydroxy-2-(2-hydroxyethylaminomethyl)-6H-pyrzolo[4,5,1-de]acridin-6-one, N-[1-[2(diethylamino)ethylamino]-7-methoxy-9-oxo-9H-thioxanthen-4-ylmethyl]formamide, N-(2-(dimethylamino)ethyl)acridine-4-carboxamide, 6-[[2-(dimethylamino)ethyl]amino]-3-hydroxy-7H-indeno[2,1-c]quinolin-7-one, and dimesna.

"Antiproliferative agents" includes antisense RNA and DNA oligonucleotides such as G3139, ODN698, RVASKRAS, GEM231, and INX3001, and antimetabolites such as enocitabine, carmofur, tegafur, pentostatin, doxifluridine, trimetrexate, fludarabine, capecitabine, galocitabine, cytarabine ocfosfate, fosteabine sodium hydrate, raltitrexed, paltitrexid, emitefur, tiazofurin, decitabine, nolatrexed, pemetrexed, nelzarabine, 2'-deoxy-2'-methylidenecytidine, 2'-fluoromethylene-2'-deoxycytidine, N-[5-(2,3-dihydro-benzofuryl)sulfonyl]-N'-(3,4-dichlorophenyl) urea, N6-[4-deoxy-4-[N2-[2(E),4(E)-tetradecadienoyl]glycylamino]-L-glycer-o-B-L-mannoheptopyranosyl]adenine, aplidine, ecteinascidin, troxacitabine, 4-[2-amino-4-oxo-4,6,7,8-tetrahydro-3H-pyrimidino[5,4-b] [1,4]thiazin-6-yl-(S)-ethyl]-2,5-

thienoyl-L-glutamic acid, aminopterin, 5-fluorouracil, alanosine, 11-acetyl-8-(carbamoyloxymethyl)-4-formyl-6-methoxy-14-oxa-1,11-diazatetracyclo(7.4.1.0.0)-tetradeca-2,4,6-trien-9-yl acetic acid ester, swainsonine, lometrexol, dexrazoxane, methioninase, 2'-cyano-2'-deoxy-N4-palmitoyl-1-B-D-arabino furanosyl cytosine, and 3-aminopyridine-2-carboxaldehyde thiosemicarbazone. "Antiproliferative agents" also includes monoclonal antibodies to growth factors, other than those listed under "angiogenesis inhibitors", such as trastuzumab, and tumor suppressor genes, such as p53, which can be delivered via recombinant virus-mediated gene transfer (see U.S. Pat. No. 6,069,134, for example).

Some specific examples of tyrosine kinase inhibitors include N-(trifluoromethylphenyl)-5-methylisoxazol-4-carboxamide, 3-[(2,4-dimethylpyrrol-5-yl)methylidene]indolin-2-one, 17-(allylamino)-17-demethoxygeldanamycin, 4-(3-chloro-4-fluorophenylamino)-7-methoxy-6-[3-(4-morpholinyl)propoxy]quinazoline, N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)-4-quinazolinamine, BIBX1382, 2,3,9,10,11,12-hexahydro-10-(hydroxymethyl)-10-hydroxy-9-methyl-9,12-epoxy-1H-diindolo[1,2,3-fg:3',2',1'-kl]pyrrolo[3,4-i][1,6]benzodiazocin-1-one, SH1382, genistein, ST1571, CEP2563, 4-(3-chlorophenylamino)-5,6-dimethyl-7H-pyrrolo [2,3-d]pyrimidinemethane sulfonate, 4-(3-bromo-4-hydroxyphenyl)-amino-6,7-dimethoxyquinazoline, 4-(4'-hydroxyphenyl)amino-6,7-dimethoxyquinazoline, SU6668, ST1571A, N-4-chlorophenyl-4-(4-pyridylmethyl)-1-phthalazine, and EMD121974.

If formulated as a fixed dose, such combination products employ the compounds of this invention within the dosage range described below and the other pharmaceutically active agent(s) within its approved dosage range. Compounds of the instant invention may alternatively be used sequentially with known pharmaceutically acceptable agent(s) when a combination formulation is inappropriate.

The term "administration" and variants thereof (e.g., "administering" a compound) in reference to a compound of the invention means introducing the compound or a prodrug of the compound into the system of the animal in

need of treatment. When a compound of the invention or prodrug thereof is provided in combination with one or more other active agents (e.g., a cytotoxic agent, etc.), "administration" and its variants are each understood to include concurrent and sequential introduction of the compound or prodrug thereof and other agents.

The term "therapeutically effective amount" as used herein means that amount of active compound or pharmaceutical agent that elicits the biological or medicinal response in a tissue, system, animal or human that is being sought by a researcher, veterinarian, medical doctor or other clinician.

The term "treating cancer" or "treatment of cancer" refers to administration to a mammal afflicted with a cancerous condition and refers to an effect that alleviates the cancerous condition by killing the cancerous cells, but also to an effect that results in the inhibition of growth and/or metastasis of the cancer.

The present invention also encompasses a pharmaceutical composition useful in the treatment of cancer, particularly cancers involving inappropriate tyrosine kinase activity, comprising the administration of a therapeutically effective amount of the compounds of this invention, with or without pharmaceutically acceptable carriers or diluents. Suitable compositions of this invention include aqueous solutions comprising compounds of this invention and pharmacologically acceptable carriers, e.g., saline, at a pH level, e.g., 7.4. The solutions may be introduced into a patient's bloodstream by local bolus injection.

When a compound according to this invention is administered into a human subject, the daily dosage will normally be determined by the prescribing physician with the dosage generally varying according to the age, weight, and response of the individual patient, as well as the severity of the patient's symptoms.

In one exemplary application, a suitable amount of compound is administered to a mammal undergoing treatment for cancer. Administration occurs in an amount between about 0.1 mg/kg of body weight up to less than about 50 mg/kg of body weight and more preferably up to about 25 mg/kg of

body weight per day, and still more preferably between about 0.5 mg/kg of body weight to about 25 mg/kg of body weight per day.

In summary, the compounds of the invention have been shown to be useful in the treatment of a variety of cancers. Furthermore, the predictive value of the *in silico* data has been confirmed with the *in vitro* data.

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitation.

Examples

Methods of synthetic chemistry, protein and peptide biochemistry, molecular biology, and pharmacology referred to but not explicitly described in this disclosure and examples are reported in the scientific literature and are well known to those skilled in the art.

Example 1

Molecules with the potential target biological activity were analyzed in a validated *in silico* assay that is based on public domain National Cancer Institute *in vitro* anti-cancer data. The molecules are first decomposed to 110 descriptors using a proprietary CHEMSASTM algorithm. This decomposition process results in a molecular data pattern of 110 variables that is then input into the *in silico* model. The output of the model is a prediction of the -Log(GI50) for the molecule(s) being analyzed against the specific cancer cell type in question i.e. breast cancer or leukemia, etc. A specific *in silico* assay was also developed for the leukemia cell line (i.e. K562) that over expresses the abnormal protein tyrosine kinase found in chronic myelogenous leukemia (CML) and P388 murine acute myelogenous leukemia (ALL). Results of the *in*

silico assay for molecular Formulas I and II in a number of cancer cell types are summarized below in Table 1.

Table 1

5	Compound	leukemia	K562(CML)*	NSCLC**	SCLC***	Colon	CNS
	Formula I/II	-5.4	-5.0	-4.9	-4.8	-5.2	-4.9
	Compound	Melanoma	Ovary	Renal	Prostate	Breast	
	Formula I/II	-4.7	-4.8	-4.8	-5.1	-4.6	

10

Note: Values in the table refer to the $-\text{Log}(\text{GI}_{50})$ as a molar concentration.

If $-\text{Log}(\text{GI}_{50}) > -4.5$ then the compound is likely to be inactive.

If $-\text{Log}(\text{GI}_{50}) > -5$ and < -4.5 then the compound is likely to have some *in vitro* activity.

15 If $-\text{Log}(\text{GI}_{50}) < -5$ then the compound is considered to have *in vitro* activity.

*K562 is a specific leukemia cell line for CML that over expresses the abnormal protein tyrosine kinase.

** NSCLC is a non small cell lung cancer

***SCLC is a small cell lung cancer

20

Example 2 - *In Vitro* data for COTI-001 (formula II)

The COTI-001 compound was tested *in vitro* on a variety of cell lines comparing its effect to a known protein tyrosine kinase inhibitor used in the treatment of certain cancers. The data shows that the COTI-001 compound

25 was comparably effective with that of GleevecTM.

For Leukemia Cell Line K562

	Formula II	Gleevec TM
Predicted	-5	-5.4
95% CI	± 0.65	± 0.65
30 Actual	-4.7	-6

For Leukemia Cell Line P388

	<u>Formula II</u>	<u>GleevecTM</u>
Predicted	-5.7	-5.4
5 95% CI	± 0.65	± 0.65
Actual	-6	-5.7

For Breast Cancer Cell Line MCF7

	<u>Formula II</u>	<u>GleevecTM</u>
10 Predicted	-5.1	-5.3
95% CI	± 0.65	± 0.65
Actual	-4.7	-4.8

Predicted: means the *in silico* predictions of $-\text{Log}(\text{GI}_{50})$.

15 Actual: means *in vitro* results.

Example 3 - In Vivo Toxicity Studies of COTI-001

Five groups of mice (3 mice per group) were administered a 50mg/kg dosage of GleevecTM. The mice were able to tolerate this dosage amount.

20 Five groups of mice (3 mice per group) were administered a 50mg/kg dosage of Formula II. This dosage was not tolerated by the mice. The mice, however, were able to tolerate a dosage of about 25mg/kg and less.

Example 4 - Synthesis of the Salt of Formula II (COTI-001)25 Intermediate 1

Intermediate 1 was prepared as shown in Scheme 2, using a similar procedure as described in European Patent Application 0225725 A1.

Synthesis of 5-Methyl-1, 4, 5, 6-tetrahydro-2-(4-nitrophenyl)pyrimidine 3

30 Anhydrous ethanol (460 mL) and sodium (0.57 g, 25 mmol, 0.1 eq) were added to a round bottom flask and stirred under argon until all of the sodium disappeared. 4-nitrobenzonitrile (38.0 g, 257 mmol, 1.0 eq) was then

added. The suspension was stirred for 15 h at room temperature under argon. 1,3-diamino-2-methylpropane (22.0 g, 249 mmol, 0.97 eq) was added. The mixture was heated to reflux for 4 days. After removal of the solvent under reduced pressure, the resulting red residue was mixed with ethyl acetate (800 mL) and extracted with 2N HCl (3 x 200 mL). The combined aqueous mixture was washed with ethyl acetate (250 mL) and the organic layer was removed. The acidic aqueous mixture was basified with solid Na_2CO_3 and then 5N NaOH until pH was 13. The emulsion was extracted with ethyl acetate (1 x 400 mL, 2 x 250 mL). The combined organic layers were washed with brine (400 mL) and then dried over Na_2SO_4 . Upon concentrating, compound **3** was obtained as a yellow solid (12.8 g, 23% yield).

Synthesis of 1-Boc-5-methyl-1, 4, 5, 6-tetrahydro-2-(4-nitrophenyl)pyrimidine
4

Compound **3** (12.9 g, 58.7 mmol, 1.0 eq), methylene chloride (600 mL) and N,N-dimethylaminopyridine (0.73 g, 6.0 mmol, 0.1 eq) were added to a 2L 3-necked round bottom flask. Di-tert-butyl dicarbonate (Boc_2O) (15.4 g, 70.4 mmol, 1.2 eq) was added to the above mixture in portions. The resulting mixture was stirred for 14 h at room temperature under argon. After removal of the solvent under reduced pressure, the red residue was purified by column chromatography (silica gel; ethyl acetate:hexanes 1:2 to 1:1 v/v) to give compound **4** (16.6 g) in 89% yield.

Synthesis of 1-Boc-5-methyl-1, 4, 5, 6-tetrahydro-2-(4-aminophenyl)pyrimidine

1

Raney Ni (19.0 g) was added to a 500 mL 3-necked round bottom flask, followed by washing with anhydrous ethanol (30 mL x 3) and then ethanol (50 mL) was added. To the suspension was added a solution of compound **4** (16.6 g, 52.0 mmol, 1.0 eq) in ethanol (230 mL). Hydrazine monohydrate (10.4 g 208 mmol, 4.0 eq) was added dropwise at 50 °C, followed by heating to 75 °C for 5 min. Upon cooling to room temperature, the catalyst was removed by suction filtration through celite. The filtrate was

evaporated to dryness, then co-evaporated with toluene twice and finally dried at 60 - 65 °C for 30 min under reduced pressure to give compound 1 (14.8 g) in 99% yield.

5 Synthesis of N,N'-Bis[4-(1,4,5,6-tetrahydro-5-methyl-2-pyrimidinyl)phenyl]-2,5-pyridinedicarboxamide dihydrochloride (Formula II)

Pyridine-2,5-dicarboxylic acid (3.88 g, 23.2 mmol, 1.0 eq), o-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU, 19.4 g, 51.1 mmol, 2.2 eq), 1-hydroxybenzotriazole hydrate (HOBt, 6.90 g, 51.1 mmol, 2.2 eq), intermediate 1 (14.8 g, 51.1 mmol, 2.2 eq) and anhydrous dimethylformamide (250 mL) were added to a 500 mL 3-necked round bottom flask under argon. With stirring, diisopropylethylamine (DIPEA, 13.2 g or 17.8 mL, 102 mmol, 4.4 eq) was slowly added, resulting in a red solution. After stirring at room temperature for 15 h, a suspension was obtained. The solvent was removed by evaporation under vacuum. The residue was further purified by column chromatography (silica gel; gradient eluants: 1.5% - 5% methanol in methylene chloride) to give the Boc-protected coupling compound as a solid (3.15 g) in 38% yield.

The Boc-protected coupling compound (3.14 g, 4.42 mmol) was mixed with a cold saturated HCl solution in methanol (105 mL) at 0 °C. The resulting reaction mixture was stirred at room temperature under argon for 1h before concentrating to approximately 50 mL and cooling down to 0 °C for 30 min. An off-white solid precipitate was collected by filtration and washed with cold MeOH and diethyl ether, followed by drying at 65 °C under high vacuum to give Formula II (1.89 g) in 74% yield.

All compounds synthesized as above were identified by ¹H NMR and Formula II was identified by ¹H NMR and MS.

Although preferred embodiments of the invention have been described herein in detail, it will be understood by those skilled in the art that variations may be made thereto without departing from the spirit of the invention.